SAMPLE COLLECTION AND PREPARATION OF PPP

Although no special preparation of the patient is required prior to sample collection by approved techniques, it is preferable that patients are not heavily exercised before blood collection. Fasting or only light non-fatty meals prior to blood collection provide samples with a desirable lower opacity.

REAGENT PREPARATION

Bring all the reagents to Room temperature prior to testing.

(a) To prepare 5.0 ml working reagent take one vial of Optiplastin-r concentrate (R1) and one vial of Optiplastin-r Diluent (R2). (b) Transfer the entire content of R1 vial into R2 vial and gently swirl the R2 vial to mix the content well. Rinse R1 vial again using 500 µl of mixture from

INR Conversion Table

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REOCCUMINT RABBIT THROMBOPLASTIN REAGENT FOR PROTHROMBIN TIME (PT) DETERMINATION

SUMMARY

The arrest of bleeding depends upon primary platelet plug formed along with the formation of a stable fibrin clot. Formation of this clot involves the sequential interaction of series of plasma proteins in a highly ordered and complex manner and also the interaction of these complexes with blood platelets and materials released from the tissues. Tissue Thromboplastin, in the presence of calcium, is an activator, which initiates the extrinsic pathway of coagulation, which includes plasma coagulation factors VII, X, V, Prothrombin and Fibrinogen. During oral anticoagulant therapy most of the Vitamin K dependent factors such as VII, IX, X, Protein C and Protein S are depressed, as also during the deficiencies of clotting factor activity which may be hereditary or acquired. Prothrombin Time determination is the preferred method for presurgical screening, as a liver function test, determination of congenital deficiency of factors II, VII, X and XIII and for monitoring of patients on oral anticoagulant therapy.

Originally, thromboplastin reagents were prepared from tissues (usually brain or placenta) of human or animal origin. Because of its varying composition and preparation, their sensitivity in monitoring oral anticoagulant therapy also varies widely. Recently, newer generation of thromboplastin reagents have been developed that are based on purified recombinant rabbit tissue factor that has been reconstituted into synthetic phospholipids. The phospholipid membranes demonstrated even minor deficiencies in factors involved in the extrinsic and common coagulation pathways. The ISI values of the reagents can be controlled for lot-to-lot consistent performance. Due to the recombinant source the reagent is free from infectious contaminants like HIV and Hepatitis.

Optiplastin-r is a novel recombinant rabbit thromboplastin for prothrombin time (PT) determination.
the R2 vial to ensure the complete transfer of the thromboplastin concentrate into the diluent. (c) Gently mix the R2 vial and keep it for 10 minutes. This is the working reagent now ready to use. (d) Mix the working reagent thoroughly before withdrawing every time for testing.

TEST PROCEDURE

Manual Method
1. Bring the working reagent vial to room temperature (20-30°C). Gently mix the content of the vial to homogenize the suspension completely.
2. Aspirate enough reagent from the working reagent vial for immediate testing requirements in a thoroughly clean and dry test tube. (Plastic test tubes are preferred).
3. Prewarm the reagent and bring to 37°C before use in test procedure (5-10 minutes may be required depending on the reagent volume to attain 37°C before testing).
4. Recap the working reagent vial and replace immediately to 2-4°C.
5. To a 1 x 75 mm tube add 0.1 ml of plasma (PPP) and place the tube in a waterbath for 3 to 5 minutes at 37°C.
6. To the tube forcibly add 0.2 ml of working reagent (prewarmed at 37°C for atleas 3 minutes) and simultaneously start a stopwatch. Shake the tube gently to mix contents.
7. Gently fill the tube back and forth and stop the stopwatch as soon as the first fibrin strand is visible and the gel clot formation begins.
8. Repeat steps 5-6 for a duplicate test on the same sample.
9. Find the average of the duplicate test values. This is the Prothrombin Time (PT).
If a coagulation instrument is being used to perform the tests, the instrument manufacturers instructions must be strictly adhered to.

CALCULATION OF RESULTS

Manual Method
The results may be reported directly in terms of the mean of the double determination of PT of the test plasma in 'seconds'.

Or as a ratio R:\[R = \frac{\text{Mean of the patient plasma PT in seconds}}{\text{Normal plasma PT in seconds}}\]

MINP for the reagent

Or as an International Normalized Ratio (INR), INR = (R)^{0.577}, where ISI = International Sensitivity Index of the reagent (Refer reagent vial label).
It is recommended by the WHO that MNPT should be established for each lot of PT reagents by each laboratory, since PT results are dependent on the combination of reagent lot, instrument and technique followed at each laboratory. Usually plasma from atleas 20 normal healthy individuals should be used to establish the MNPT. The average of such PT results in seconds = MNPT.

EXPECTED VALUES

Normal values using OPTIPLASTIN-v are between 8 - 13 seconds on Opticlot-4 (Photo Optical coagulation instrument). Between manual and Photo Optical instrument results, a variation of 1-2 seconds may be expected. For Opto mechanical instruments, it is recommended that each laboratory must establish their own normal range. It is mandatory that each laboratory must establish its own MNPT for each lot of OPTIPLASTIN-v.

Oral Anticoagulant Therapeutic range: INR = 2.0 - 3.5

REMARKS

(1) It is recommended that controls with known factor activity should be run simultaneously with each test series to validate test run. (2) Incorrect mixture of blood and tri-sodium citrate, insufficient prewarming of plasma and reagent, contaminated glassware etc. are potential source of errors. (3) Since the PT test functions correctly only at 37± 0.5°C, temperature of all equipment must be calibrated daily. (4) Clotting time of patients on anticoagulant therapy depends upon the type and dosage of anticoagulant and also the time lag between the specimens collected and the last dose. (5) Turbid, icteric, lipemic or grossly hemolyzed samples may generate erroneous PT results. (6) Glasswares and cuvettes used in the test should be scrupulously clean and free from even traces of acids/alkalis or detergents. (7) Plasma samples held at 4-8°C may undergo 'cold activation' leading to a marked shortening of the PT. (8) The PT may be prolonged during acute inflammatory conditions which are accompanied by increase in fibrinogen levels and also by agents such as antistaminics, barbiturals, phenobarbital, caffeine, oral contraceptives and vitamin K. The PT may be prolonged by corticosteroids, EDTA, oral contraceptives, aspirin, clofibrate, erythromycin, ethanol, tetracycline, aspirin and anticoagulants such as heparin and warfarin. (9) It is important that each laboratory expresses the results in terms of INR for patients on oral anticoagulant therapy for the clinician to adjust the dosage based on INR. (10) Since the test uses platelet poor plasma, each laboratory must calculate the necessary force and time required during centrifugation to yield the PPP. Contamination of plasma with excess platelets could lead to false low levels of some of the factors. (11) Inhibitors such as lupus anticoagulant may interfere with the prothrombin time and result for example in INRs that do not reflect the exact degree of anticoagulation. (12) Hirudin, Oxalate and other direct thrombin inhibitors in therapeutic dose result in prolonged prothrombin times. (13) Some blood collection tubes may contain Mg ions, which have been shown to interfere with recombinant thromboplastin. (14) Homogenisation of OPTIPLASTIN-v reagent suspension before use is important to achieve accurate and consistent results.

WARRANTY

This product is designed to be described as the label and package insert. The manufacturer disclaims any implied warranty of use and sale for any other purpose.

BIBLIOGRAPHY


- Interpolate the reading intersecting the ratio row (R), as obtained for the patient sample with the reagent ISI value from the ISI column.
- MNPT: Each laboratory should establish its own MNPT as per the procedure given in the OPTIPLASTIN-v package insert. It is recommended to approximately include equal number of males and females in the age group of 18-45 by following the sample collection procedures diligently and carefully excluding out donors on interfering medications.
- The individuals included for establishing MNPT should represent the population routinely tested in the laboratory, with blood collection methods, testing techniques and instrumentation routinely used in the specific laboratory.
- In case of major changes in type of reagents, instrumentation, blood collection techniques or anticoagulant, MNPT should be reestablished. MNPT should at least be verified for different lots of the same reagent.

MONITORING ORAL ANTICOAGULANT THERAPY USING ISI AND INR

- Oral anticoagulant drugs derived from coumarin and sometimes indanediones are widely used in the Prophylaxis and treatment of thrombotic disorders. Adjustment of the dose of these drugs is periodically required to ensure that adequate and not excessive degree of anticoagulation is achieved.
- The ICH and the international committee for hemostasis and thrombosis have agreed to the reporting of Prothrombin time (PT) results based on the ISI (International Sensitivity Index) of the thromboplastin reagents and INR (International Normalised Ratio).
- OPTIPLASTIN-v Reagent is assigned an ISI value by calibration against an international preparation, which by definition has a preassigned sensitivity (ISI). OPTIPLASTIN-v Reagent is assigned an ISI value based on the method as recommended by the WHO. The ISI value assigned to OPTIPLASTIN-v Reagent defines its comparative slope or relative sensitivity as compared to the IRP/standard product.
- In general the lower the ISI value of a thromboplastin reagent the more sensitive it is.
- INR is calculated from the PT ratio (R) using a thromboplastin with a known ISI. The INR may be interpreted as the prothrombin time ratio that would have been obtained if the same plasma had been tested using the WHO international standard rabbit brain thromboplastin (IRP RBT/05).
- The use of INR's enables direct comparison to be made between all results on patient plasmas regardless of interlab variations or reagent in question.

The INR is calculated as INR = (R)^{0.577} where ISI = Lot specific ISI for the reagent

\[
\text{INR} = \left( \frac{\text{Patient PT}}{\text{Mean normal PT}} \right)^{0.577}
\]

Mean normal PT = Mean of the normal range that is specifically determined by each user laboratory for each lot of thromboplastin reagent with specific instrument and techniques routinely used for patient testing.

Example:
Patient PT result = 21 seconds
MNPT = 11 seconds
ISI of reagent = 1.0

\[
R = \frac{21.0}{11.0} = 1.9
\]

INR = (1.9)^{0.577} = 1.9

Recommended Therapeutic Range for Oral Anticoagulant Therapy

- Prophylaxis of venous thrombosis (High risk surgery)
- Treatment of venous thrombosis (High risk surgery)
- Prevention of systemic embolism - Tissue heart valves
- Prevention of systemic embolism - Mechanical prosthetic valves
- prevention of recurrent myocardial infarction

2.0-3.0

2.5-3.5

- Mechanical prosthetic valves (High risk)
- Vasculitis
- Mechanical prosthetic valves (Low risk)